

Seasonal variation in testicular and fat-body weight and plasma testosterone and androstenedione concentration in the lizard *Cordylus polyzonus* (Sauria: Cordylidae)

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Seasonal variation in testicular and fat-body weight and plasma testosterone and androstenedione concentration of the lizard *Cordylus polyzonus* is described. Testicular recrudescence commenced in autumn (April), reaching a peak in early spring (September) and regression followed during mid-spring to early autumn (October–March). The onset of testicular recrudescence coincided with decreasing ambient temperature and photoperiod and increasing rainfall. Fat-body weight was small during most of the testicular recrudescence phase, with an increase commencing in spring (September). A peak in fat-body weight was reached during late spring – midsummer (October–December) and followed by a progressive decline to baseline values in winter. Plasma testosterone and androstenedione concentrations followed a bimodal annual pattern, a first peak occurring in late summer/early autumn (February/March) and the second in spring (September/October). The first peak coincided with the onset of testicular recrudescence and the second with maximum testicular weight.

Seisoenale variasie in testes- en vetliggaamgewig en plasmatestosteroon- en -androstiendioonkonsentrasies van die akkedis *Cordylus polyzonus* word beskryf. Testikulêre groei neem 'n aanvang in herfs (April), 'n piek word bereik in die vroeë lente (September) en regressie volg vanaf middel lente–vroeë herfs (Oktober–Maart). Die aanvang van testikulêre groei val saam met 'n daling in omgewingstemperaturen fotoperiode en 'n toename in reënval. Vetliggaamgewig is laag met die aanvang van testikulêre groei en 'n toename begin in die lente (September). 'n Piek in vetliggaamgewig word bereik tydens laat lente–middel somer (Oktober–Desember), gevolg deur 'n progressiewe afname na laagste waardes in die winter. Plasmatestosteroon- en -androstiendioonvlakke vertoon 'n bimodale seisoenale patroon, met 'n eerste piek in die laat somer/vroeë herfs (Februarie/Maart) en 'n tweede in die lente (September/Oktober). Die eerste piek val saam met die aanvang van testikulêre groei en die tweede piek met maksimum testikulêre gewig.

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The family Cordylidae is considered poorly-known with regard to life history patterns (Dunham, Miles & Reznick 1988) and detailed reproductive data are available for males of only two species, *Cordylus polyzonus* and *Pseudocordylus melanotus* (Van Wyk 1990; Flemming 1993). The study on *C. polyzonus* reports seasonal variation in testis and femoral gland activity (Van Wyk 1990) and the one on *P. melanotus* testis and fat-body activity (Flemming 1993). For other members of the family Cordylidae information on male reproduction consists primarily of anecdotal observations (FitzSimons 1943; Loveridge 1944; Branch 1988).

In the studies on male reproductive activities of *C. polyzonus* and *P. melanotus* (Van Wyk 1990; Flemming 1993) an effort has been made to correlate testicular activity with seasonal changes in climatic factors. Reproductive cycles in reptiles are generally correlated with climatic variables such as photoperiod, rainfall and temperature (Duvall, Guillet & Jones 1982; Marion 1982; Licht 1984; James & Shine 1985). Of these, temperature is considered the most important climatic factor controlling and regulating reproductive cycles of temperate zone reptiles (Duvall *et al.* 1982; James & Shine 1985). Indications are that temperature plays an important role in this regard in *C. polyzonus* and *P. melanotus* (Van Wyk 1990; Flemming 1993). Information on other external factors (physical and social) controlling reproduction in reptiles, as well as internal ones (neural and hormonal) are scarce (Licht 1984; Crews & Gans 1992), and lack for cordylids.

Involvement of testicular steroids has been illustrated in

reptilian spermatogenesis, sexual behaviour and in stimulating secondary reproductive organs such as the epididymis, the sexual segment of the kidney and femoral glands (Dufaure & Chambon 1978; Licht 1984; Moore & Lindzey 1992). Such studies are, however, uncommon and indicate great diversity in the physiological role of sex steroids in reptiles (Licht 1984; Moore & Lindzey 1992). According to Licht (1984) data on secretory patterns of these steroids may be of considerable value in understanding their physiology.

Seasonal cycles of lipid utilization and deposition in reptiles usually reflect periods of energy shortage and surplus (Derickson 1976; Pond 1978). In many lizards the bulk of lipids are stored in abdominal fat bodies, and seasonal change in fat-body lipid content reflects changes in total lipid content (Derickson 1976; Droge, Jones & Ballinger 1982). Seasonal changes in reptilian fat bodies are generally related to winter maintenance and/or reproduction (Derickson 1976; Selcer 1987).

An intraspecific study on *C. polyzonus* females showed widely separated populations to exhibit similar timing of reproductive events (Van Wyk 1989; Flemming & Van Wyk 1992). Such intraspecific comparisons of geographically separated populations are considered to be valuable in quantification of geographic variation in life history traits and, if they include detailed ecological data, may offer tests of specific predictions from life history theory (Dunham *et al.* 1988).

In the present paper seasonal variation in testicular and fat body weight and plasma testosterone and androstenedione

concentrations of *C. polyzonus* males from the south-western Cape Province is investigated. The testicular changes are related to environmental variables and that of male *C. polyzonus* previously studied from the Orange Free State (Van Wyk 1990).

Materials and Methods

Collecting and autopsy procedure

The study was conducted over a period of one year (February 1986–January 1987) at Saldanha Bay (33°00'S/17°56'E) in the south-western Cape Province, South Africa. The study area falls in a semi-arid region with a typical winter rainfall pattern and a dry summer period (Schulze & McGee 1978). Mean monthly minimum and maximum air temperatures, total monthly rainfall and annual photoperiod for the study area are presented in Figure 1 (from Flemming & Van Wyk 1992).

Males were collected monthly and anaesthetized with diethyl ether within 24 h of capture. Blood samples obtained by decapitation were centrifuged and the plasma stored at -20°C until assayed. Body weight was determined to the nearest 0,01 g and snout-vent length (SVL) measured to the nearest 0,1 mm using vernier calipers. Testes and fat bodies were excised immediately after death and weighed to the nearest 0,1 mg.

Radioimmunoassays

Plasma testosterone and androstenedione concentrations were determined by radioimmunoassays at the Department of Chemical Pathology, Medical School, University of Cape Town, South Africa. Plasma testosterone determinations were performed on duplicate plasma aliquots (0,05 – 0,20 ml) using a procedure similar to that of Bennet (1989). TRK 402 tritiated testosterone (Radiochemical Centre, Amersham, UK) was used as tracer and a highly specific antiserum raised in rabbits against testosterone-3-carboxymethyl-oxime-bovine serum albumin (Millar & Kewley 1976). Cross-reaction of the antiserum with all major naturally occurring steroids was < 0,01%, except for dihydrotestosterone for which it was 5,1%.

Owing to low remaining plasma volumes after the

testosterone radioimmunoassay, 0,05 ml plasma from each male was pooled into a single monthly sample, mixed on vortex and the androstenedione radioimmunoassay performed on 0,2 ml plasma aliquants in duplicate. The procedure followed was similar to that described by Lindeque, Skinner & Millar (1986). TRK 454 tritiated androstenedione (Radiochemical Centre, Amersham, UK) was used as tracer, and a highly specific antiserum raised in rabbits against androstenedione-7 α -carboxyethyl-thioether-bovine serum albumin (bio-Yeda, Israel). Cross-reaction of the antiserum with all naturally occurring steroids was < 0,6%, except for testosterone and dehydroepiandrosterone for which it was 3% in both cases.

Extraction efficiency as suggested by the recovery of the tracer was 92–96% for the testosterone assay and 90–95% for the androstenedione assay. The intra-assay coefficient of variance (determined by an assay of replicate aliquots of plasma controls) was 7,4% for testosterone and 8,3% for androstenedione. The between-assay-variance for the same samples was 9,2% for testosterone and 10,1% for androstenedione. Sensitivity of the assays was 156 pmol/l. Serial dilutions of plasma samples gave displacement curves parallel to the standard curve.

Statistical analyses

Analysis of covariance (ANCOVA) with SVL as covariate was used to determine whether testicular and fat-body weight were significantly affected by body size. Analysis of variance (ANOVA) was used to analyse seasonal variation in reproductive parameters, followed by Tukey's multiple range test to determine significant differences in monthly samples (Sokal & Rohlf, 1981). The Pearson's product-moment correlation test was used to determine if any correlation existed between testes weight and hormonal concentrations. Partial correlation and stepwise multiple regression analyses were used to investigate covariation between reproductive condition and environmental variables (Sokal & Rohlf 1981). The mean testicular weight for each monthly sample was the dependent variable, and total monthly rainfall, maximum and minimum environmental temperatures and photoperiod the independent variables. $P < 0,05$ was considered as significant and all means are followed by ± 1 standard error.

Results

Testicular weight changes

The smallest male with enlarged testes measured 93 mm SVL, therefore only males with a SVL > 95 mm were used in the present study; mean SVL was $101,1 \pm 0,44$ mm ($n = 77$). ANCOVA revealed that testicular weight was not significantly affected by variation in male SVL ($F_{1,77} = 0,01$; $p > 0,05$). Testicular weight changes were distinctly seasonal (ANOVA; $F_{11,65} = 18,15$; $p < 0,05$; Figure 2a), with an increase commencing in mid-autumn (April). A peak was reached in early spring (September), thereafter a significant decrease followed in October and baseline values were recorded during spring to early autumn (November–March). The testicular cycle was well synchronized with the ovarian cycle, with peak testicular weights reached just before ovulation in October (Flemming & Van Wyk 1992).

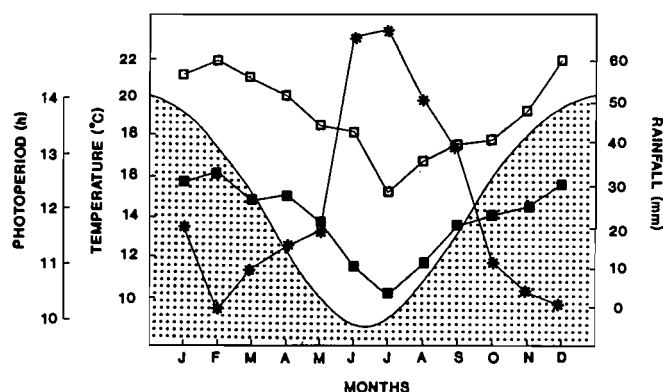


Figure 1 Mean monthly maximum temperatures (open squares), monthly minimum temperatures (black squares), total monthly rainfall (asterisks), and photoperiod (shaded area) at the study area (Saldanha Bay; 33°00'S/17°56'E) for 1986.

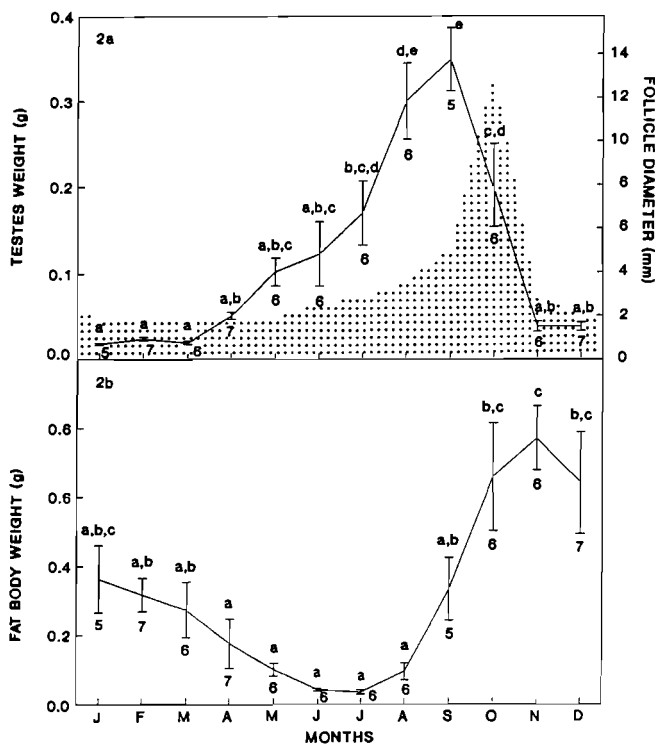


Figure 2 Variation in (a) testicular weight and (b) fat-body weight in *Cordylus polyzonus* males. Values are presented mean \pm 1SE. The shaded area represents the female reproductive cycle of *C. polyzonus* females in the study area (after Flemming & Van Wyk 1992). Numbers below values indicate sample sizes and values with different superscripts are significantly different (Tukey's Multiple Range Test; $p < 0,05$ per comparison).

Fat-body weight changes

ANCOVA revealed that fat-body weight ($F_{1,77} = 0,27$; $p > 0,05$) was not significantly affected by variation in male SVL. Fat-body weight showed significant seasonal variation (ANOVA; $F_{11,65} = 9,52$; $p < 0,05$; Figure 2b), with an increase commencing in spring (September), during which time testes were largest. A peak was reached during October–December and fat-body weight decreased progressively thereafter, reaching lowest values during the mid-autumn/winter (June/July) testicular recrudescence phase.

Plasma androgen concentrations

Plasma testosterone concentration showed a bimodal annual trend (ANOVA; $F_{11,65} = 16,85$; $p < 0,05$; Figure 3), with a peak occurring in February, declining thereafter during March and April to low early winter (May/June) concentrations (first testosterone phase). Concentrations increased from July to reach a second annual peak in September/October, declining thereafter to low concentrations in January (second testosterone phase). The second testosterone phase showed a significant correlation with testicular weight ($r = 0,63$; $p < 0,05$).

Plasma androstenedione concentrations paralleled those of mean monthly testosterone concentrations ($r = 0,91$; $p < 0,05$), with two annual peaks evident (ANOVA; $F_{1,12} = 359,3$; $p < 0,05$; Figure 3). The first peak occurred during February/March and a second during August/September.

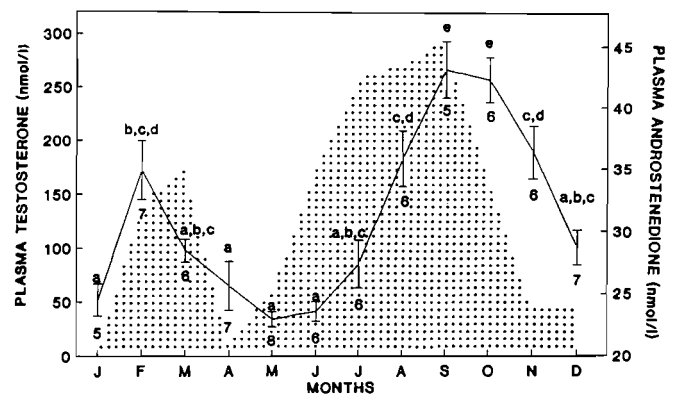


Figure 3 Variation in plasma testosterone and androstenedione concentrations in *Cordylus polyzonus* males. Testosterone concentrations are presented mean \pm 1SE. Monthly values for androstenedione (shaded area) are for pooled samples. For testosterone concentrations, numbers below values indicate sample sizes and values with different superscripts are significantly different (Tukey's Multiple Range Test; $p < 0,05$ per comparison).

Climatic correlates

Onset of testicular growth coincided with decreasing ambient temperature and photoperiod and increasing rainfall. Multiple regression analysis indicated that of the climatic variables included as independent variables, maximum temperature was the best predictor of seasonal variation in mean monthly testicular weight (testicular weight = $0,91 - 0,4$ maximum temperature; $r = 0,76$; $p < 0,05$), explaining 59% of the variation. The correlation between mean testicular weight and maximum temperature was negative and significant ($r = -0,77$; $p < 0,05$), as was the correlation with minimum temperature ($r = -0,67$; $p < 0,05$). The correlation between mean testicular weight and rainfall was positive and significant ($r = 0,58$; $p < 0,05$).

Discussion

C. polyzonus males from the south-western Cape Province (present study) exhibited autumn/winter testicular recrudescence, followed by regression in summer. A similar pattern was reported for *C. polyzonus* males from the Orange Free State (Van Wyk 1990). This pattern is in contrast with that observed for most temperate zone male squamates where reproductive activity is exhibited during the warmer months, with testicular recrudescence in spring and maximum testicular activity in summer (Fitch 1970; Duvall *et al.* 1982; Licht 1984; Guillette & Casas-Andreu 1987). Several male squamates, however, have an autumn/winter breeding pattern similar to that of *C. polyzonus* (Guillette & Casas-Andreu 1987; Van Wyk 1990). As was reported in *C. polyzonus* from the Orange Free State (Van Wyk 1990), *C. polyzonus* from the south-western Cape Province exhibited considerable synchrony in timing of male and female reproductive processes.

Lipid resources in most reptiles are apparently used for winter maintenance and/or reproduction (Derickson 1976; Selcer 1987). In male *C. polyzonus*, fat bodies were small during most of the testicular recrudescence phase in winter and an increase commenced during early spring, during

which time maximum testicular activity (this study; Van Wyk 1990) and mating (FitzSimons 1943; Fitch 1970) occurred. Food availability is considered the ultimate factor determining whether or not lipids are stored (Derickson 1976) and it is therefore likely that during spring and early summer, *C. polyzonus* males had adequate dietary intake to sustain metabolic activities and also to build fat reserves. The midsummer – winter decrease in fat-body weight possibly reflects a period of lowered dietary intake, or simply the balance between energy used for reproductive versus other processes.

In most reptiles, seasonal changes in plasma androgen concentrations are monophasic and peak concentrations coincide with peak spermatogenesis and mating (Licht 1984; Moore & Lindzey 1992). In *C. polyzonus* males peak plasma testosterone and androstenedione concentrations were indeed observed during spring when maximum spermatogenic activity (Van Wyk 1990) and mating (FitzSimons 1943; Fitch 1970) is reported to take place. Androgen involvement has been illustrated in mating and spermatogenesis, as well as stimulating secondary reproductive organs in other reptiles (Dufaure & Chambon 1978; Licht 1984; Moore & Lindzey 1992). The pattern in *C. polyzonus* males is different from that observed in some reptiles (especially those exhibiting postnuptial spermatogenesis) where plasma testosterone and spermatogenic cycles do not follow the same pattern, and low sex steroid concentrations occur during the breeding season (see Licht 1984; Lance 1984; Licht, Breitenbach & Congdon 1985).

A second annual peak in plasma testosterone and androstenedione concentrations was, however, evident in *C. polyzonus*. This peak coincided with the onset of spermatogenesis, which occurs in late summer/early autumn (Van Wyk 1990). A biphasic pattern of seasonal concentrations in plasma androgens has been recorded in some reptiles, one peak usually coinciding with maximum spermatogenesis and the other with mating (Weil & Aldridge 1981; Lance 1984), or just after hibernation (Naulleau, Fleury & Boissin 1987), or with minimal activity in seminiferous tubules (Licht *et al.* 1985; Kuchling, Skolek-Winnisch & Bamberg 1981). In some reptiles exhibiting a monophasic plasma androgen profile, the testicular androgen concentrations, however, are biphasic (Arslan, Lobo, Zaidi, Jalali & Qazi 1978; Bourne & Seamark 1975; Bourne, Taylor & Watson 1986; Courty & Dufaure 1979; Bona-Gallo, Licht, MacKenzie & Loftis 1980).

Androgen involvement in the regulation of the onset of spermatogenesis has been proposed in reptiles on the basis of high plasma and/or testicular androgen concentrations at the time (Licht 1984). However, involvement of other steroids or even peptide hormones in the initiation of spermatogenesis has also been proposed. Lance (1984), for instance, reviewed the endocrinology of reproduction in male reptiles and proposed that in chelonians and crocodilians, initiation of spermatogenesis is dependent on FSH and does not require high androgen concentrations, but that maturation of spermatocytes does.

Temperature is considered the most important environmental factor in controlling and regulating reproductive cycles of temperate zone reptiles, either by direct action, or

owing to the existence of a temperature threshold to facilitate a photoperiod response (Marion 1982; Duvall *et al.* 1982; Licht 1984; James & Shine 1985). *C. polyzonus* males from both the Orange Free State (Van Wyk 1990) and south-western Cape Province became reproductively active during a period of declining ambient temperature and photoperiod, suggesting that one or both of these factors act as environmental cues in the onset of testicular growth. During the onset of the reproductive cycle in *C. polyzonus* males, rainfall increased in the south-western Cape Province and decreased in the Orange Free State (Van Wyk 1990), suggesting that this environmental variable does not act as a primary cue stimulating gonadal growth in males, as has been previously suggested for females (Flemming & Van Wyk 1992). Although rainfall is considered an important cue in tropical reptiles (Duvall *et al.* 1982; Fitch 1982; James & Shine 1985), it is not generally considered a proximal cue in temperate reptiles (Duvall *et al.* 1982). There is some evidence that low temperature affects gonadal recrudescence in turtles (Mendonça 1987; Ganzhorn & Licht 1983), but more work on the role of decreasing temperature and/or photoperiod as cues for stimulating reproductive activity in reptiles is needed.

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